Electronic Supplementary Information (ESI)

Interactions between Circulating Nanoengineered Polymer Particles and Extracellular Matrix Components *In Vitro*

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Supplementary Figures

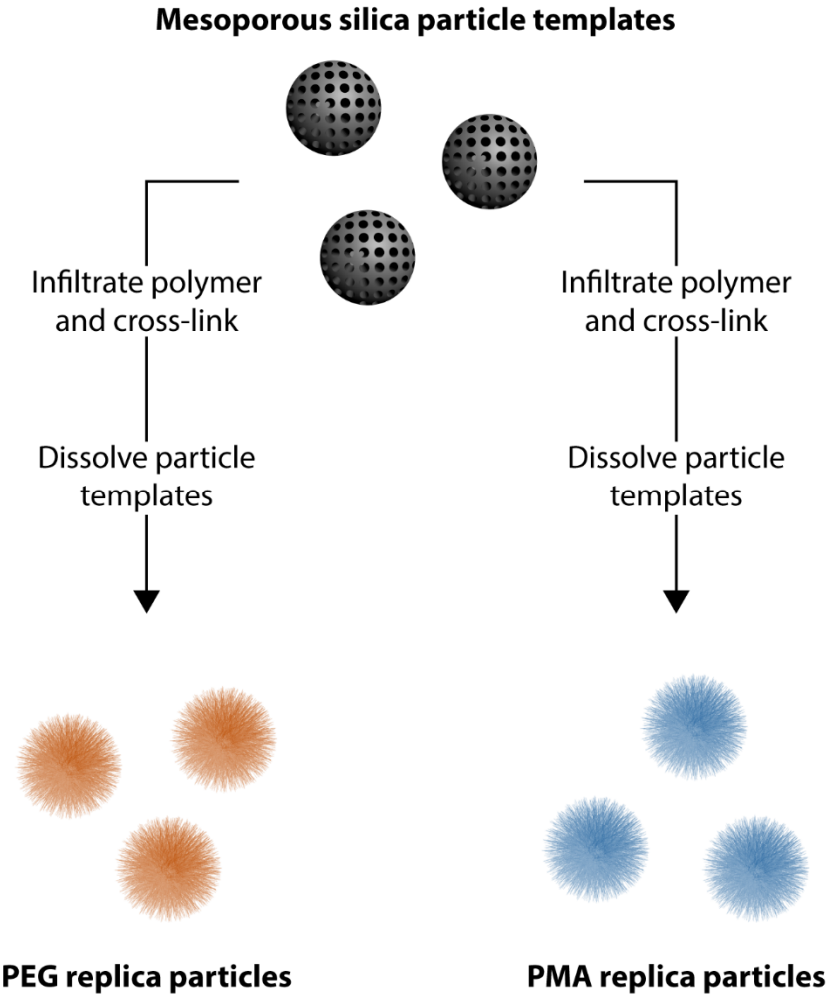


Fig. S1. Preparation of polymer replica particles through mesoporous silica replication.[1] The same batch of mesoporous silica particle templates was used when preparing both PEG and PMA replica particles. Additional details are available in Fig. S2 and S3.

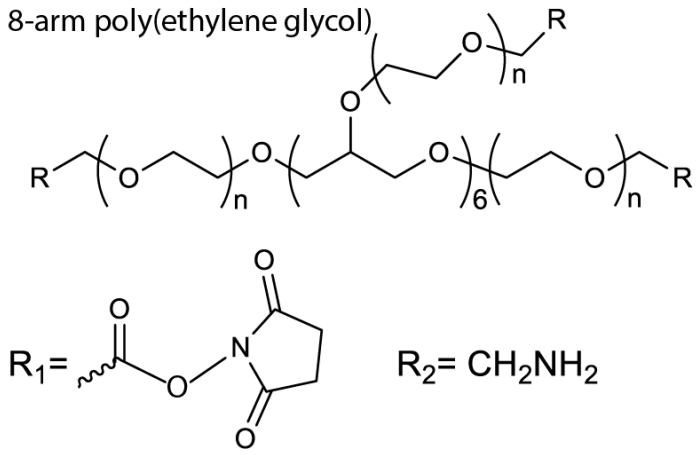


Fig. S2. Molecular structures of the 8-arm-PEGs used for the assembly of PEG replica particles.

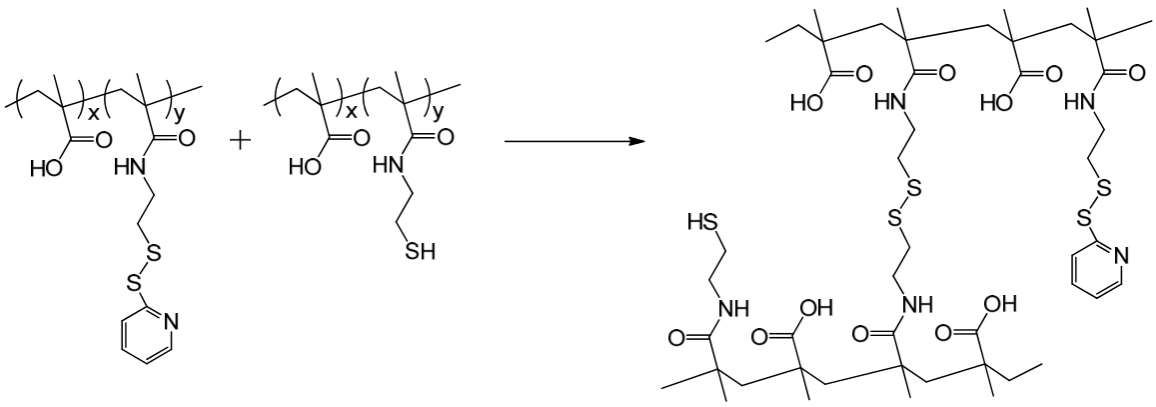


Fig. S3. Molecular structures of polymers and scheme of cross-linking strategy used to prepare PMA replica particles.

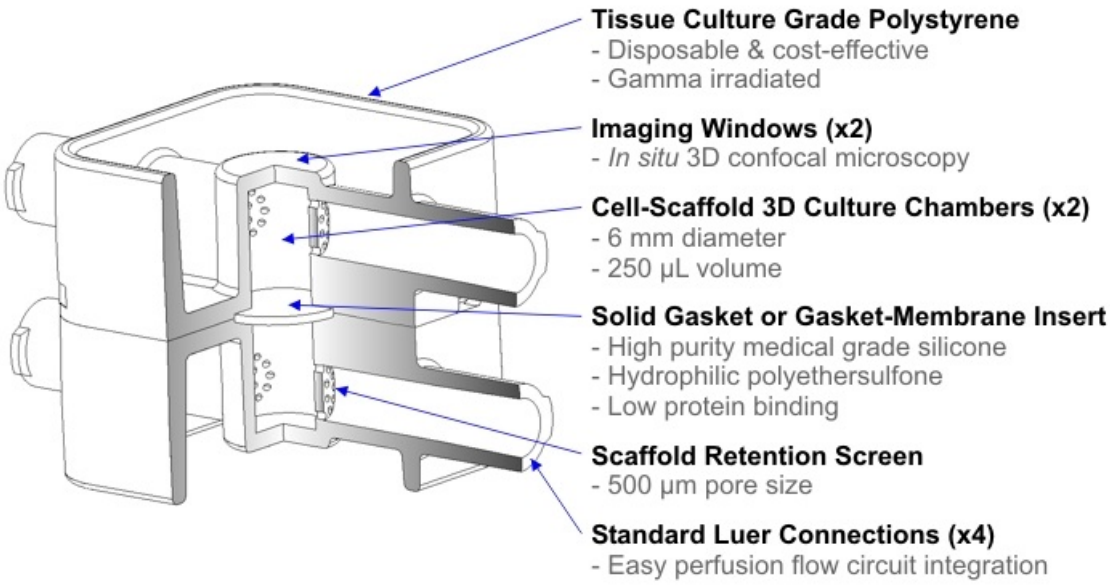


Fig. S4. Specifications and dimensions (supplied by the manufacturer) of the 3DKUBE perfusion chamber.

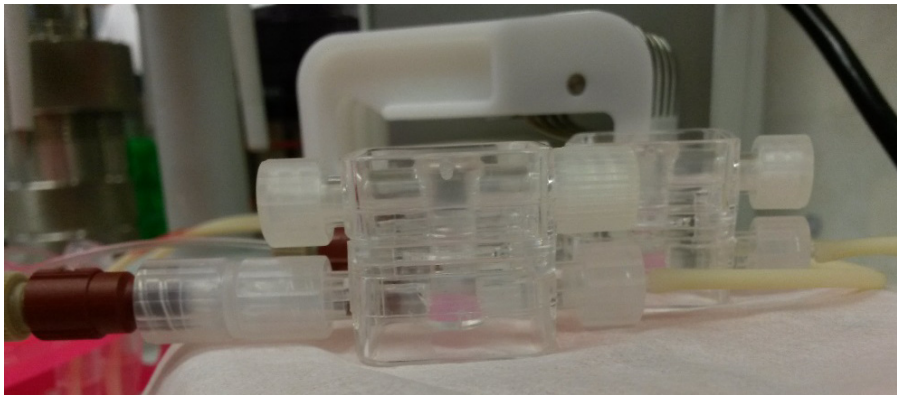


Fig. S5. Photograph of 3DKUBE. The peristaltic pump can be seen in the background.

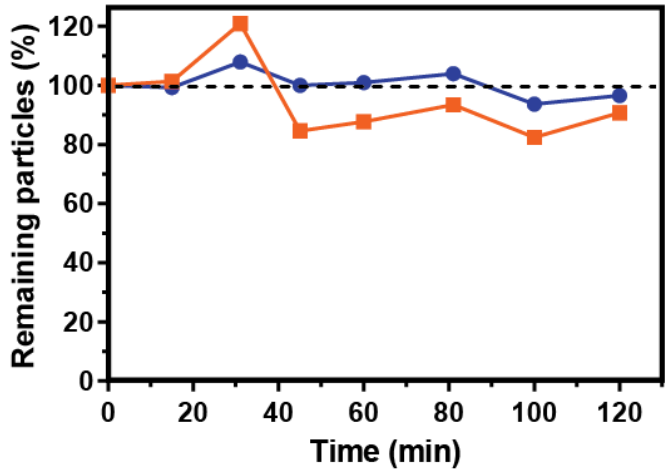
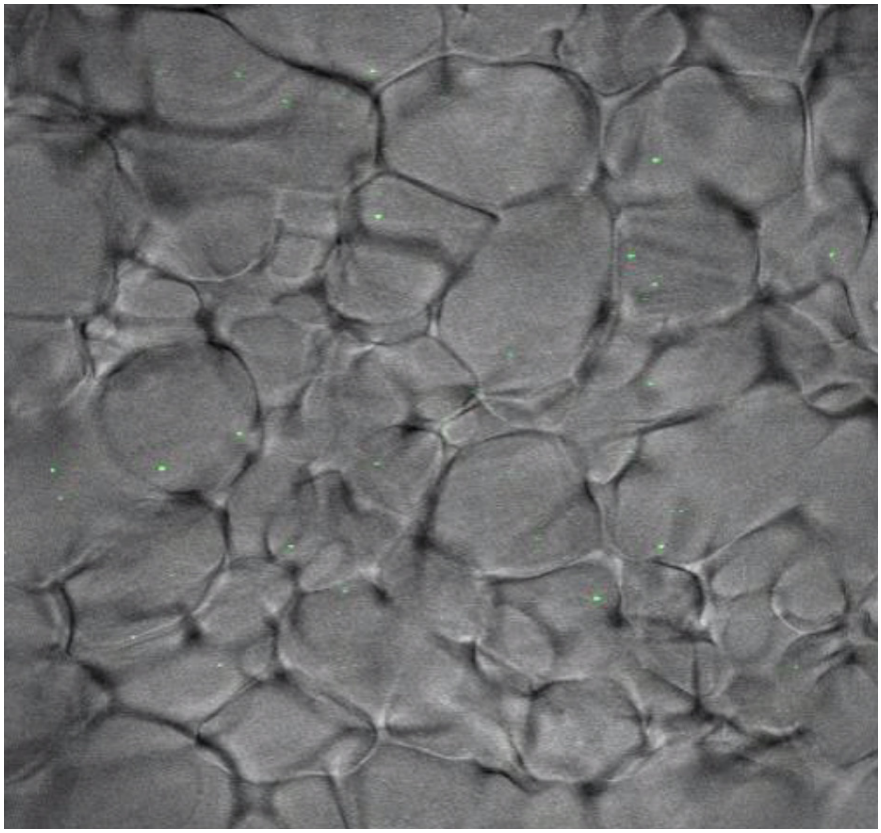


Fig. S6. Change in concentration of PEG (orange circles) and PMA (blue squares) replica particles over time in the empty flow system (without any extracellular matrix components present) monitored via flow cytometry.



Video S1. Single frame from video sequence showing mobile PEG particles (green) trapped in HA gel (bright-field) under the steady flow conditions used during the *in vitro* circulation studies. The flow direction is from right to left. The video is available online on the publisher's webpage together with the article.

Supplementary Experimental Details

Preparation of PEG particles. MS particles (~500 nm) were synthesized based on a previously reported method[3] and PEG particles were prepared via a MS templating approach.[3,2] Briefly, 6 mg of MS particles were washed with phosphate buffer (100 mM, pH 8) and incubated in 480 μL of 8-arm-PEG-NH_2 solution (5 mg mL^{-1} in phosphate buffer) with constant shaking for at least 8 h. Subsequently, the particles were washed three times with phosphate buffer. The pellet was dispersed in 400 μL of 8-arm-PEG-NHS solution (2 mg mL^{-1} in phosphate buffer) containing 10 μg of AF488-NHS (1 mg mL^{-1} in DMSO) and incubated for at least 2 h, where PEG was crosslinked and formed networks in the MS particles. After three washing cycles with water, MS templates were removed with a 2 M HF/8 M NH_4F solution (pH ~5). *Caution! HF is highly toxic. Extreme care should be taken when handling HF solution and only small quantities should be prepared*. The resultant PEG particles were washed three times with water and resuspended in 500 μL of water.

Preparation of PMA particles. PMA_{PDA} was synthesized via EDC-mediated amide bond formation between the carboxyl groups of PMA and the amine groups of PDA, based on a previously reported method.[4] In a typical experiment, a PMA solution (360 mg of 30 wt.% solution, 1 equiv. of MA) was diluted into 5 mL of phosphate buffer (0.1 M, pH 7.2). The resulting solution was incubated with EDC (57.5 mg, 0.3 equiv.) with stirring for 15 min. Subsequently, PDA (33.4 mg, 0.15 equiv.) was added to the mixture and the pH was adjusted to 7.2. The reaction was allowed to proceed overnight. The resulting mixture was placed inside a dialysis membrane (molecular weight cut-off 3500 Da, Thermo Fisher), dialyzed extensively against water, filtered with a 0.2 μm syringe filter, and isolated via lyophilisation. The degree of thiol functionalization was characterized by measuring the absorbance of the released pyridine-2-thione (λ_{max} = 343 nm), and then quantified from a calibration curve of PDA, which corresponded to 10 mol% modification. PMA_{SH} particles were fabricated via thiol-disulphide exchange cross-linking according to a previously published method.[5,6] Briefly, 6 mg of amine-modified MS templates were incubated with 1.8 mg of PMA_{PDA} (5 mg mL^{-1} in 100 mM acetate buffer, pH 5) under constant shaking for at least 5 h. Subsequently, the polymer-loaded templates were isolated by centrifugation and washed three times with acetate buffer. The pellet was dispersed in 600 μL of thiolated PMA (PMA_{SH}) solution (1 mg mL^{-1} in acetate buffer) and incubated overnight (15 h). Here, PMA_{SH} was freshly prepared by the incubation of PMA_{PDA} with DTT for 15 min, followed by purification with a NAP-5 Sephadex column to obtain PMA_{SH} solution. The PMA-loaded

particles were washed three times and labelled with AF488-maleimide dye by incubating the particles with 10 μL of AF488-Maleimide dye in 200 μL of phosphate buffer (50 mM, pH 7.2) overnight. After three washing cycles with water, the templates were dissolved with a 2 M HF/8 M NH_4F solution (pH ~5). *Caution! HF is highly toxic. Extreme care should be taken when handling HF solution and only small quantities should be prepared.*

Particle characterization. Transmission electron microscopy (TEM) analysis was carried out with a Philips CM120 BioTWIN instrument operated at 120 kV. Fluorescence microscopy images were taken using an Olympus IX71 inverted fluorescence microscope. A Malvern Zetasizer Nano ZS was used to obtain the zeta potential. Particle counting was performed on an Apogee Microflow cytometer. A Nikon A1R+ laser scanning confocal microscope was used for confocal microscopy. Additional characterization of this particle system (incl. super-resolution microscopy and atomic force microscopy) has been performed previously.[3]

Hyaluronic acid (HA) cryogel fabrication. HA cryogels were prepared according to a previously published method.[7] Briefly, HA cryogels were prepared by EDC-mediated zero-length crosslinking of the HA polymer chains. 45 mg of HA were dissolved in water at 4 °C. Stock solution of EDC (0.4 M) and NHS (0.5 M) were prepared in water and added in a 2:1 molar ratio to achieve a final HA concentration of 5 wt.% with crosslinking degree of 30% per disaccharide repeating unit. The precursor solution was vortexed for 20 s and cast into the chambers of a preassembled mould. The mould consisted of two microscope slides separated by a 1 mm thick acrylic polymer spacer. The filled mould was then placed in a circulating cooling bath at –12 °C for 72 h. Cryogels were thawed in water, removed from the glass mould, and stored at room temperature (21-23 °C) in water.

ECM gel and HA gel characterization. ECM gel was characterized based on a previously published method.[8] Briefly, ECM gels were thawed overnight at 4 °C and 6 μL of ECM gel were injected into chemotaxis 3D slides and incubated at 37 °C, 5% CO_2 for 30 min. Gels were fixed using 2.5% glutaraldehyde solution for 40 min at room temperature (21-23 °C) and blocked for 24 h at 4 °C with PBS containing 1% BSA. Samples were washed with 1% BSA and incubated with primary antibody (anti-collagen IV antibody, 1:100) for 72 h at 4 °C. The gels were then washed three times with PBS, incubated with secondary antibody (goat-anti-rabbit AF488 IgG, 1:200) for 48 h at 4 °C, and washed with PBS before imaging. For labelling of HA gels with Rhodamine B, EDC (0.4 M) and NHS (0.5 M) were

mixed in a 2:1 molar ratio in PBS. The gels were dehydrated using a lint-free tissue and rehydrated in ~50 µL of EDC/NHS solution. After 25 min incubation at room temperature (21-23 °C) the gels were washed in PBS, rehydrated in a Rhodamine B solution (~100 µg ml^{-1}) and incubated overnight at 4 °C. Before imaging, the HA gels were washed at least three times with PBS to remove non-reacted dye. Images were taken on a Nikon confocal microscope using a 40× water immersion objective (1.15 NA) or a 60× oil immersion objective (1.4 NA).

Preparation of ECM/HA gel filled 3DKUBE chamber. *Half-filled chamber:* For experiments with ECM gels, a stock of ECM gel was thawed overnight at 4 °C. The next day, a chilled (4 °C) pipette tip was used to pipette 60 µL of ECM gel into a chilled (4 °C) 3DKUBE chamber. After addition of the ECM gel, four 3D insert PS scaffolds were immersed in the liquid ECM gel to confer additional stability to the gel during flow experiments. Before immersion, the PS scaffolds were soaked in ethanol and washed three times in PBS to remove any air bubbles in the scaffold. The 3DKUBE chambers were then put in a humidified chamber and incubated at 37 °C for 1 h to allow for gelation. *Fully filled chamber:* For experiments with HA gels, HA cryogels were prepared as described above. Four HA gel discs, cut using a biopsy punch (6 mm diameter), were placed on top of each other into the 3DKUBE chamber.

Flow setup. The flow setup consisted of a 1.7 mL microcentrifuge tube, a flow chamber (an assembled 3DKUBE), a peristaltic pump (Ismatec-Reglo Digital MS-CA4/12-100) and peristaltic pump tubing (PharMed, ID 0.76 mm). Before the experiment, the tubing was thoroughly rinsed for 20 min with water and PBS. The 3DKUBE chamber containing the ECM/HA gel was completely filled with PBS. The 3DKUBE experiments were run in "independent mode", meaning that a silicon gasket was used to separate the two chambers in a fully assembled 3DKUBE unit (Figure S1 and S2). The unit was then connected to the tubing in a drop-to-drop fashion to avoid introduction of air bubbles. Before the experiment, the 3DKUBE was flushed with PBS for 15 min at a flow rate of 70 µL min^{-1} to ensure an even distribution of PBS. In the meantime, a tube containing 5×10^8 particles in 600 µL was prepared and samples were taken to determine the initial particle concentration, taking the dilution of the fluidic system into account. The total volume of the flow setup was 1.2 mL which corresponds to the blood volume of a mouse (20 g body weight).[9] Once the particle-containing tube was in the flow path, the open-loop system was switched to the closed-loop configuration to allow for particle circulation. Samples were taken at designated time points

and analysed via flow cytometry (Apogee A50 Micro) to follow the particle distribution over time in relation to initial particle concentration. After the experiments the system was flushed for 30 min using PBS to remove unbound particles. The ECM/HA gels were then removed from the chamber and imaged via confocal and fluorescence microscopy (Nikon A1R+ and Olympus IX71) to evaluate the degree of trapped particles.

References

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